SPORICIDAL EFFICACY OF METHYL BROMIDE IN DECONTAMINATION OF A POROUS AND A NON-POROUS SURFACE

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ABSTRACT

Current gaseous and vaporized sporicidal decontaminants vary widely in terms of their toxicology, materials compatibility, penetrability, methods of generation, parameters for achieving efficacy and other characteristics. Methyl bromide (MeBr) is a gaseous fumigant and is known to be a broad-spectrum biocidal agent. In the past year or so, the sporicidal efficacy of MeBr against Bacillus anthracis has been confirmed based upon a limited set of screening tests. The primary objective of this study is to provide U.S. Government agencies with a comprehensive assessment of the sporicidal efficacy of MeBr. In this study, efficacy of MeBr against contaminated coupons of glass (hard surface) and carpet (porous surface) coupons contaminated with spores of avirulent (NNR1Δ1) strain of Bacillus anthracis is reported. The results show that the efficacy of MeBr is dependent on a number of parameters, such as the titer challenge level, presence of organic burden, and the type of surface being decontaminated with 6-log kill achieved on carpet at 30^{o}C in 24 hours at 50,000 ppm.

INTRODUCTION

In October 2001, several letters containing anthrax spores were sent though the U.S. Postal Service to recipients in government and private-sector buildings. The attacks resulted in 23 human anthrax infections, five of which proved fatal [(1)]. As a result of this intentional release of *B. anthracis*, several post offices, mailrooms in government buildings, and private office buildings were contaminated with *B. anthracis* spores [(2,3,4)].

At the time of the 2001 anthrax letters, there was no EPA approved building fumigants for the decontamination of *B. anthracis* spores. In order to help direct the initial building cleanup the EPA conducted a literature review of decontamination methods for *B. anthracis* spores (Spotts Whittney EA et al. 2003). The first building to be decontaminated was the Hart Senate office building, using a combination of HEPA vacuuming, surface decontamination with sodium hypochroite and finally fumigation with gaseous chlorine dioxide. The other contaminated buildings have finally been deemed safe after more than two and a half years and hundreds of millions of dollars spent using the similar methods employed in Hart Senate office building (Shane S. 2002).

Our work has focused on providing information regarding other methods for the decontamination of *B. anthracis* spores. The work presented here demonstrates the efficacy of MeBr against two coupon types contaminated with spores of avirulent (NNR1Δ1) strain of *B. anthracis.* The results show that the efficacy of MeBr is dependent on a number of parameters, such as CT, the titer challenge level, presence of organic bio-burden, and the type of surface being decontaminated.

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METHODS

Avirulent spores of the Bacillus anthracis NNR1Δ1 were prepared according to (Turnbull et al ?). Spores were diluted to approximately 2 x10^8 cfu/ml in 0.5% fetal bovine serum. Round borosilicate glass coupons (13 mm x 2.7 mm thick) were obtained from Quark Enterprises Inc. (Vineland, NJ). Square carpet coupons (13 mmx 13mm) were cut using high-pressure water knife from 100% olefin carpet, force weight 20Q2 manufactured by Shaw Industries, Inc (Dalton, GA) (Figure 1).

Five replicate coupons of each material were spotted with a 50 µl aliquot containing 2x10^8 spores/ml and dried 2 hours prior to placement in the fumigation chamber. Methyl bromide gas (Sigma St. Louis, MO) collected directly from the headspace of a 1-lb lecture bottle using a gas tight syringe was injected into the fumigation chamber via the top septum port. Methyl bromide gas mixed prior to sample removal for GC analysis. Fumigation chambers were placed in an incubator at 30°C or 37°C during the 24 or 48-hour incubation period, and relative humidity recorded. Another sample was removed for GC analysis immediately before the chamber was aerated.

Viable spores were recovered by 10-minute ultra-sonic water bath treatment (Branson Ultrasonics, Danbury, CT) in 10mls of buffer peptone water followed by 2 minutes vortexing. Spores were serially diluted to an appropriate titer values and plated on typtic soy agar followed by incubation at 37°C for up to 72 hours. Colonies were counted and log kill determined compared to unfumigated controls.

Quantitative methyl bromide analysis was carried out on a Hewitt Packard 6890 gas chromatograph fitted with a 30m Supelco (St. Louis, MO) Supel-Q plot column 0.53mm internal diameter. Samples run isocratically, 50°C Inlet/oven with helium as the carrier/make-up gas at 12.9 psi. Splitless injections of 1µl were run with no purge. Signals detected using micro-electron capture detector.

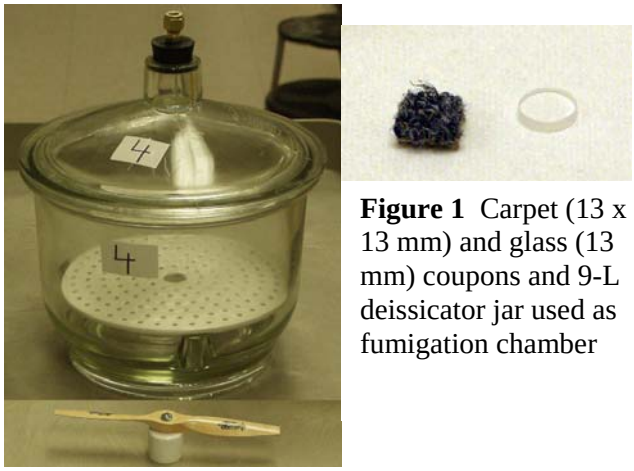

Figure 1 Carpet (13 x 13 mm) and glass (13 mm) coupons and 9-L deissicator jar used as fumigation chamber

RESULTS AND DISCUSSION

Neither organic burden nor coupon type had a significant effect on spore recovery (data not shown). Organic Burden did however greatly affect the decontamination efficacy of methyl

bromide depending on the material being tested. Only a limited effect was seen on a porous surface (carpet), where even at 5% serum, 70% of the original efficacy remained. As opposed to a non-porous surface (glass), where efficacy rapidly decreased leaving only 0.02% of the original at 5% serum (Figure 2).

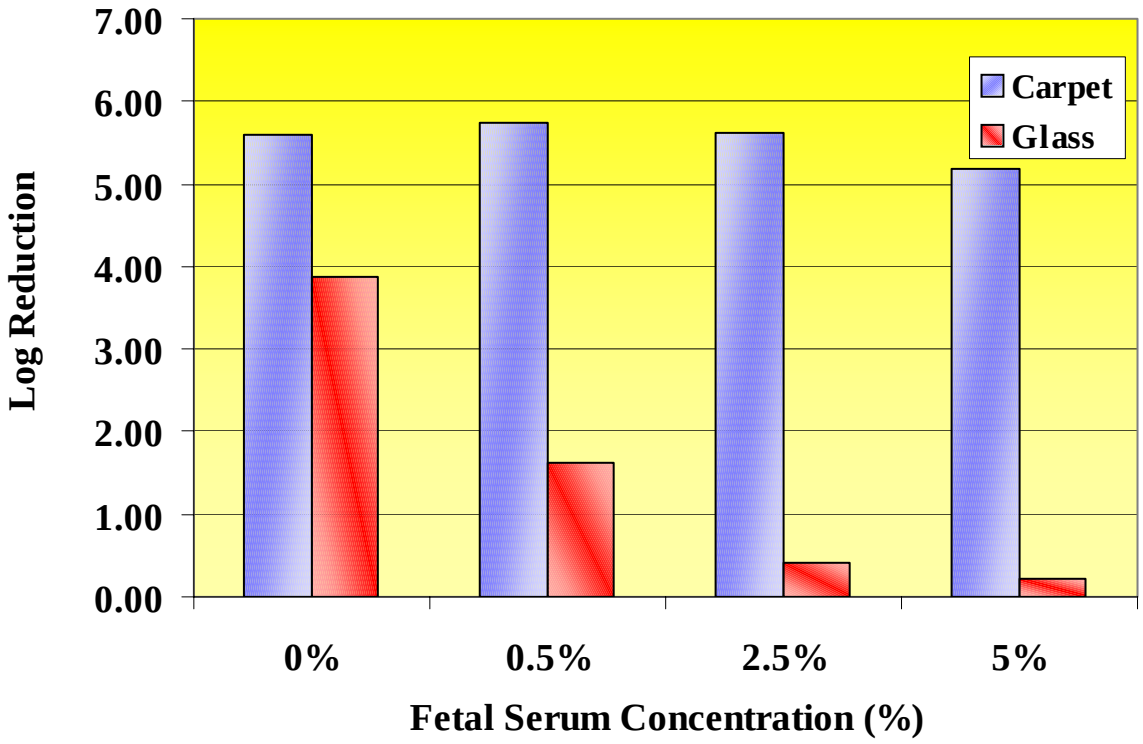

Figure 2 Effect of serum on efficacy of methyl bromide. Five replicate coupons of each type were inoculated with 50-µL aliquot containing 10^8 spores. Coupons were fumigated for 24 hours, 37°C at 50,000 ppm methyl bromide.

Methyl bromide was far more efficacious on carpet coupons compared to glass, under all conditions tested. The disparity in efficacy increased at higher gas concentrations. At 50,000 ppm methyl bromide more than 99% of the spores remained viable on glass, compared to less than 1% on carpet.

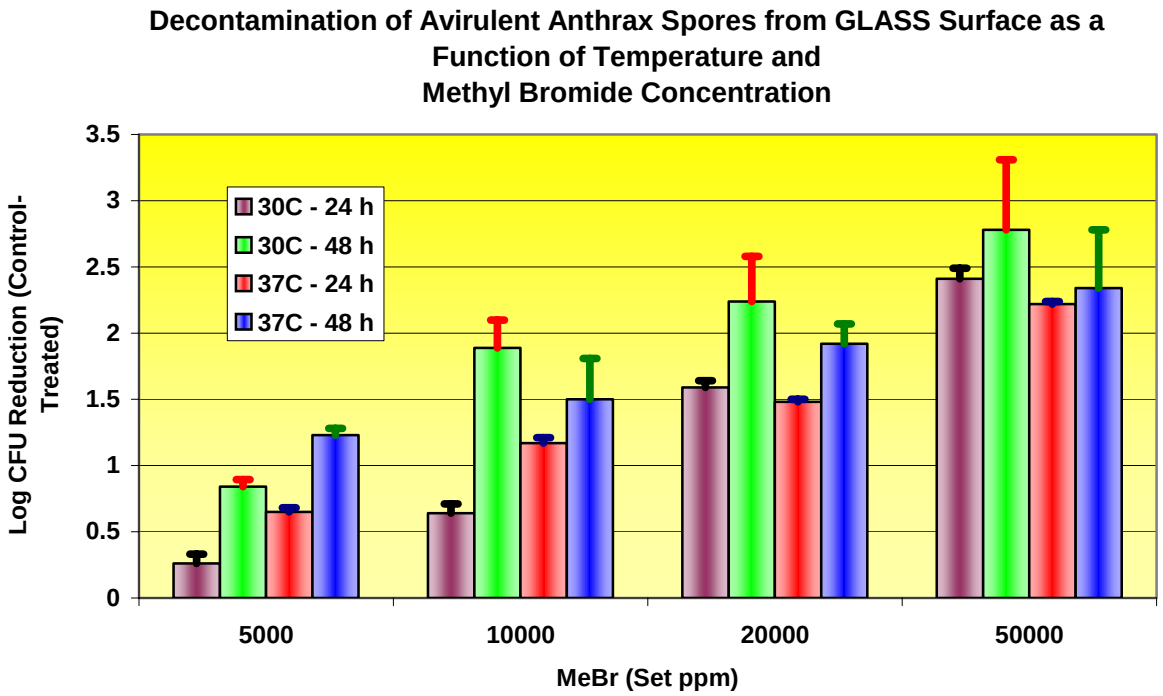

Figure 3 Determination of concentration, time, and temperature on efficacy of methyl bromide on non-porous glass. Five replicate coupons of each type were inoculated with 50-µL aliquot containing 10^7 spores.

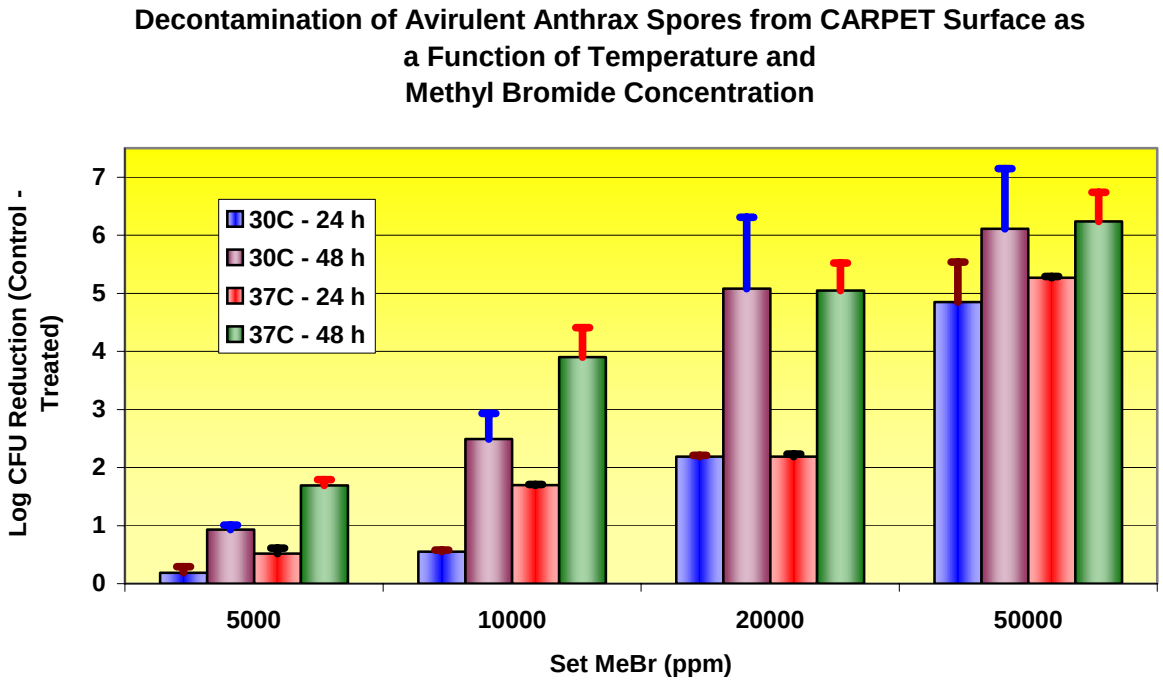

Figure 4 Determination of concentration, time, and temperature on efficacy of methyl bromide on porous carpet. Five replicate coupons of each type were inoculated with 50-µL aliquot containing 10^7 spores.

Time and temperature equally improved methyl bromide efficacy by half a log on glass. A 1.8 log increase efficacy was seen for carpet with the majority (1.25 log) related to increasing time to 48 hours. Sterilization was not seen at any methyl bromide concerntation tested. Concentrations higher than 50,000 ppm methyl bromide could not be reliably attained using our current set-up.

Although not initially identified by the EPA as an effective sporicidal agent following the October 2001 anthrax letters. Our work has demonstrated the efficacy of methyl bromide as a sporicidal fumigant, capable of a 6-log reduction of avirulent *B. anthracis* spores in 24 hours at 30^{o}C on carpet. Increasing both the temperature and time of exposure improves efficacy.

The sporicidal efficacy of methyl bromide is largely dependent on both bioburden and the surface being decontaminated. Surprisingly, non-porous glass was more difficult than porous carpet to decontaminate. This however, may be due to the presence of bioburden and the methodology employed during testing. A cohesive drop containing spores suspended in 5% serum was placed on the coupon and allowed to dry. On glass the spores would settle with the serum drying as a protective shield. The carpet fibers wick away the drop allowing for a partitioning of the spores away from the bioburden, thereby increasing their vulnerability.

REFERENCES

1. Jernigan, D. B., P. L. Raghunathan, et al. (2002). "Investigation of bioterrorism-related anthrax, United States, 2001: epidemiologic findings." Emerg Infect Dis 8(10): 1019-28.

2. Teshale, E. H., J. Painter, et al. (2002). "Environmental sampling for spores of Bacillus anthracis." Emerg Infect Dis 8(10): 1083-7.

3. Dull, P. M., K. E. Wilson, et al. (2002). "Bacillus anthracis aerosolization associated with a contaminated mail sorting machine." Emerg Infect Dis 8(10): 1044-7.

4. Sanderson, W. T., R. R. Stoddard, et al. (2004). "Bacillus anthracis contamination and inhalational anthrax in a mail processing and distribution center." J Appl Microbiol 96(5): 1048-56.

5. Spotts Whitney, E. A., M. E. Beatty, et al. (2003). "Inactivation of Bacillus anthracis spores." Emerg Infect Dis 9(6): 623-7.

6. Shane, S. (2002). Cleanup of anthrax will cost hundreds of millions of dollars. Baltimore sun. Baltimore